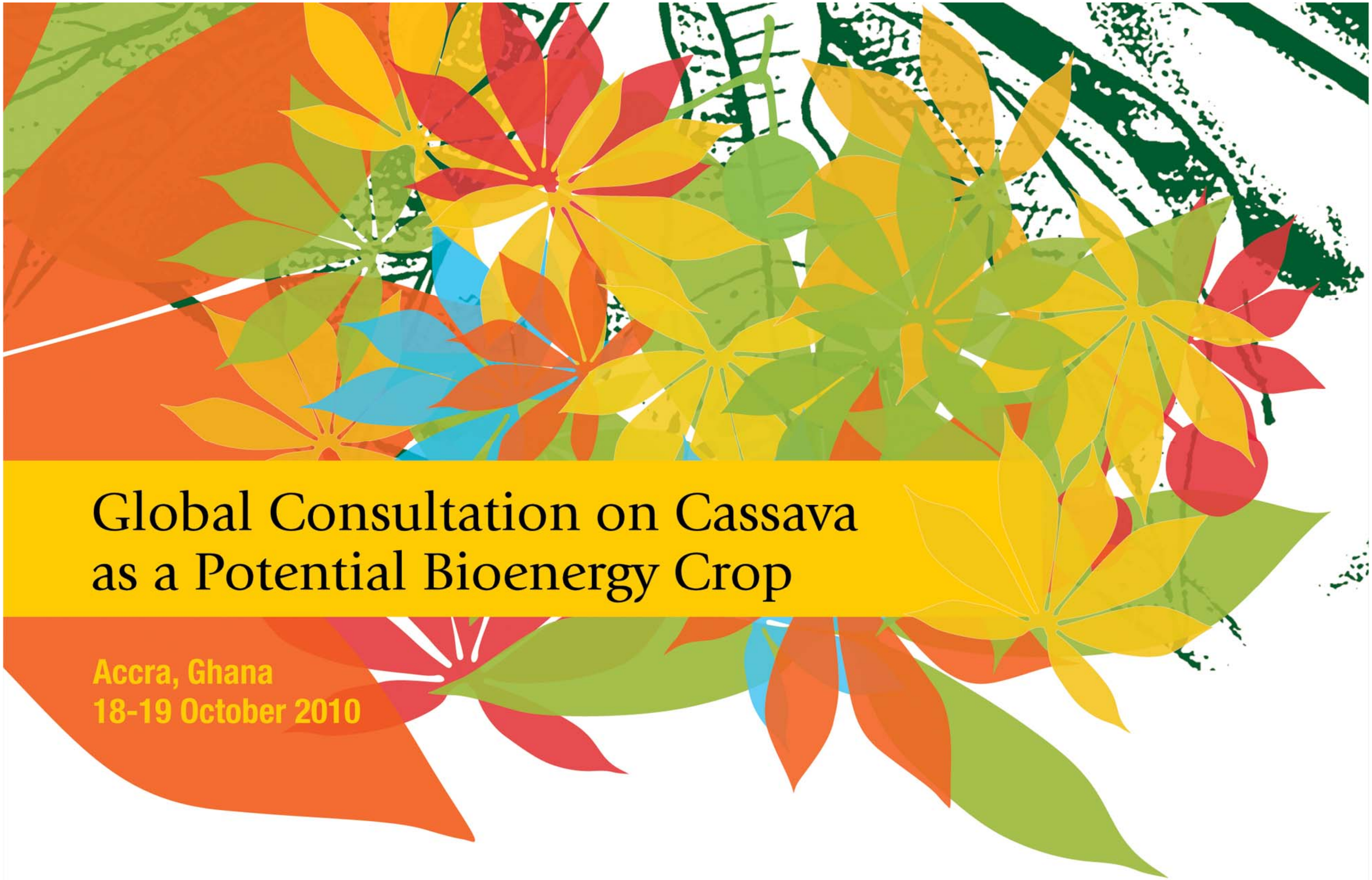

Global Consultation on Cassava as a Potential Bioenergy Crop

Accra, Ghana
18-19 October 2010

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Cassava breeding potential for bioethanol

Becerra López-Lavalle, L.A. , Dufour, D., Sánchez, T. and H. Ceballos

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Outline

- *Introduction*
- *High, stable and reliable productivity*
- *Novel traits*
- *Processing methods X root quality interactions*
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Cassava origin

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Cassava modern production

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Main uses of Cassava

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Main uses of Cassava

Chicken factory

Dry chips for animal feed

Animal feedstock

Near Hanoi, Vietnam

Pressed cake

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Main uses of Cassava

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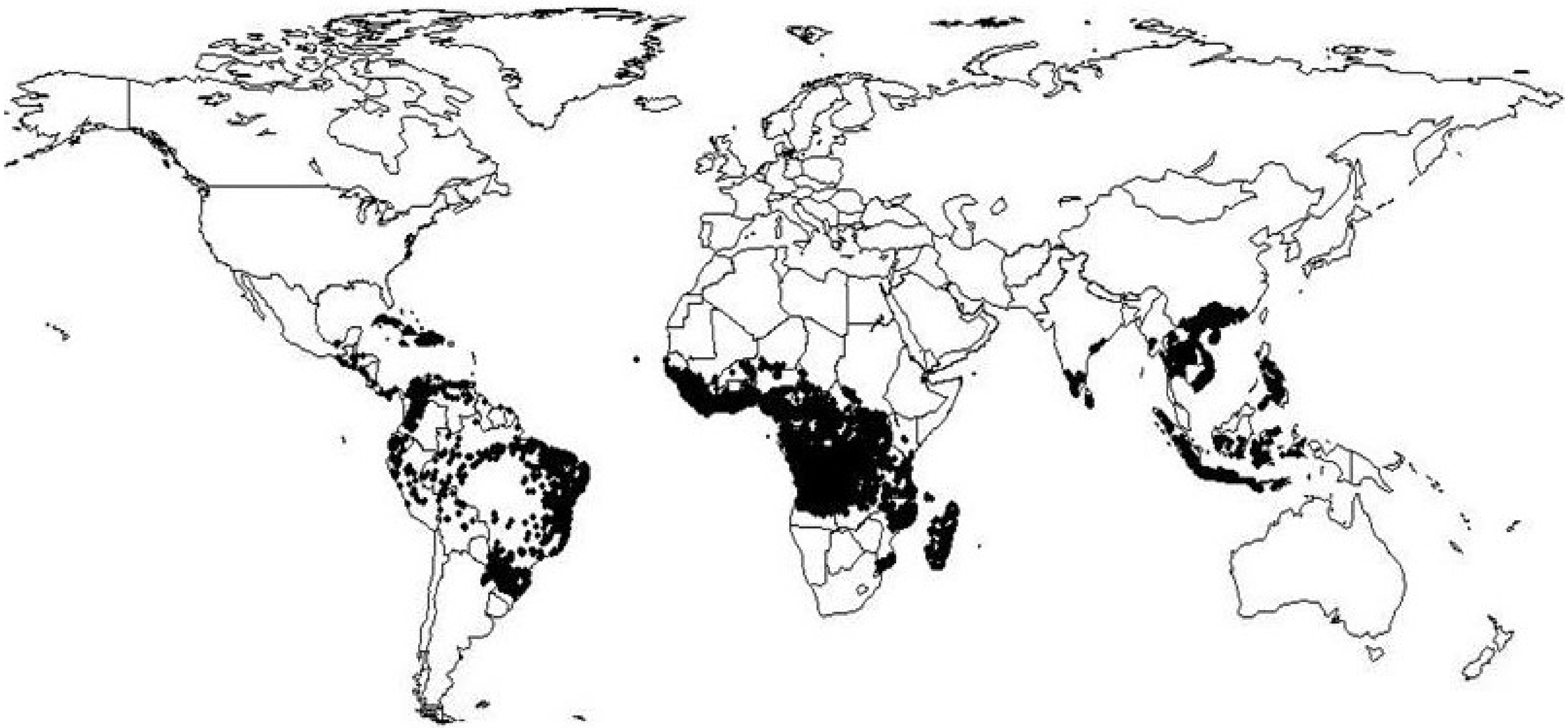
Tropical/Sub-tropical crop



Main cassava production regions in the world

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Crop Potential

South-China 5

Breeding successfully increased fresh-root (FR) productivity & dry-matter (DM) content. We now need <u>STABLE</u>-DM contents

SM 1433-4

84 t/ha FR in a 9.5 ha commercial field (~25 t/ha DM)

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Crop Potential

The case of “watery” roots for ethanol

	Fresh root yield (t/ha)	Dry matter content (%)	Dry matter yield (t/ha)
At two location: Codazzi (Cesar) and Barrancas (Guajira)			
SM 2775-2	53.8	32.1	17.3
SM 2775-4	35.3	35.9	12.7
At five location: Patalito, Sto Thomas & Molinero (Atlantico), La Union (Sucre) and Chinu (Cordoba)			
SM 2775-2	37.3	30.7	11.5
SM 2775-4	27.1	36.9	10.0

Crop Potential

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[illegible] (Atlantico), La Union [illegible]			
SM 2775-2	37.3	30.7	11.5
SM 2775-4	27.1	36.9	10.0

High Dry Matter content does not seems critical to ethanol production

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Cassava "Novel" traits

Amylose-free ("waxy") starch mutation

- *Amylose is difficult to degrade*
- *Amylose-free starch should cost less to convert into ethanol*

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Discovery of an Amylose-free Starch Mutant in Cassava (*Manihot esculenta* Crantz)

HERNÁN CEBALLOS,*,†,‡ TERESA SÁNCHEZ,† NELSON MORANTE,† MARTIN FREGENE,† DOMINIQUE DUFOUR,†,§ ALISON M. SMITH,⊥ KAY DENYER,⊥ JUAN CARLOS PÉREZ,† FERNANDO CALLE,† AND CHRISTIAN MESTRES§

Centro Internacional de Agricultura Tropical (CIAT), Apdo Aéreo 6713, Cali, Colombia, Universidad Nacional de Colombia-Palmira Campus, Centre de Coopération Internationale en Recherche Agronomique pour le Développement. (CIRAD), 73 rue Jean-François Breton, TA B-95/16, 34398 Montpellier Cedex 5, France, and John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, U.K.

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Starch/Stärke **59** (2007) 549–556 DOI 10.1002/star.200700631

Vivek Sharma
Kent D. Rausch
Mike E. Tumbleson
Vijay Singh

Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Comparison Between Granular Starch Hydrolyzing Enzyme and Conventional Enzymes for Ethanol Production from Maize Starch with Different Amylose: Amylopectin Ratios

The effect of enzyme treatments (granular starch hydrolyzing and conventional enzymes) and different amylose: amylopectin ratios of maize starch on ethanol production was evaluated. For starch treatments, amylose: amylopectin ratios were prepared by mixing commercially available Hylon VII (70% amylose and 30% amylopectin) and Amioca (~100% amylopectin) starches. For maize treatments, waxy, high amylose and regular dent hybrids were used to represent varying amylose: amylopectin ratios. All hydrolyses followed by fermentations were conducted at 15% solids content. Differences were observed in ethanol yields among granular starch hydrolyzing and conventional enzymes. Differences also were observed in ethanol yields between different amylose: amylopectin ratios for pure starch and maize samples. For starch samples, *final* ethanol concentrations varied from 2.2 to 9.1% (v/v) for fermentation with granular starch hydrolyzing enzyme and from 6.7 to 9.3% (v/v) for conventional enzymes. Higher ethanol concentrations were observed for Amioca starch for both enzymes. For maize samples, final ethanol concentrations were highest for waxy maize for both granular starch hydrolyzing (8.2%, v/v) and conventional (8.2%, v/v) enzymes. Lowest ethanol concentrations were observed for high-amylose maize samples for granular starch hydrolyzing (6.3%, v/v) and conventional (5.2%, v/v) enzymes.

Keywords: Starch hydrolysis; Fermentation; Amylose; Amylopectin; Ethanol, Granular starch hydrolyzing enzyme; Conventional enzyme; Dry grind maize process

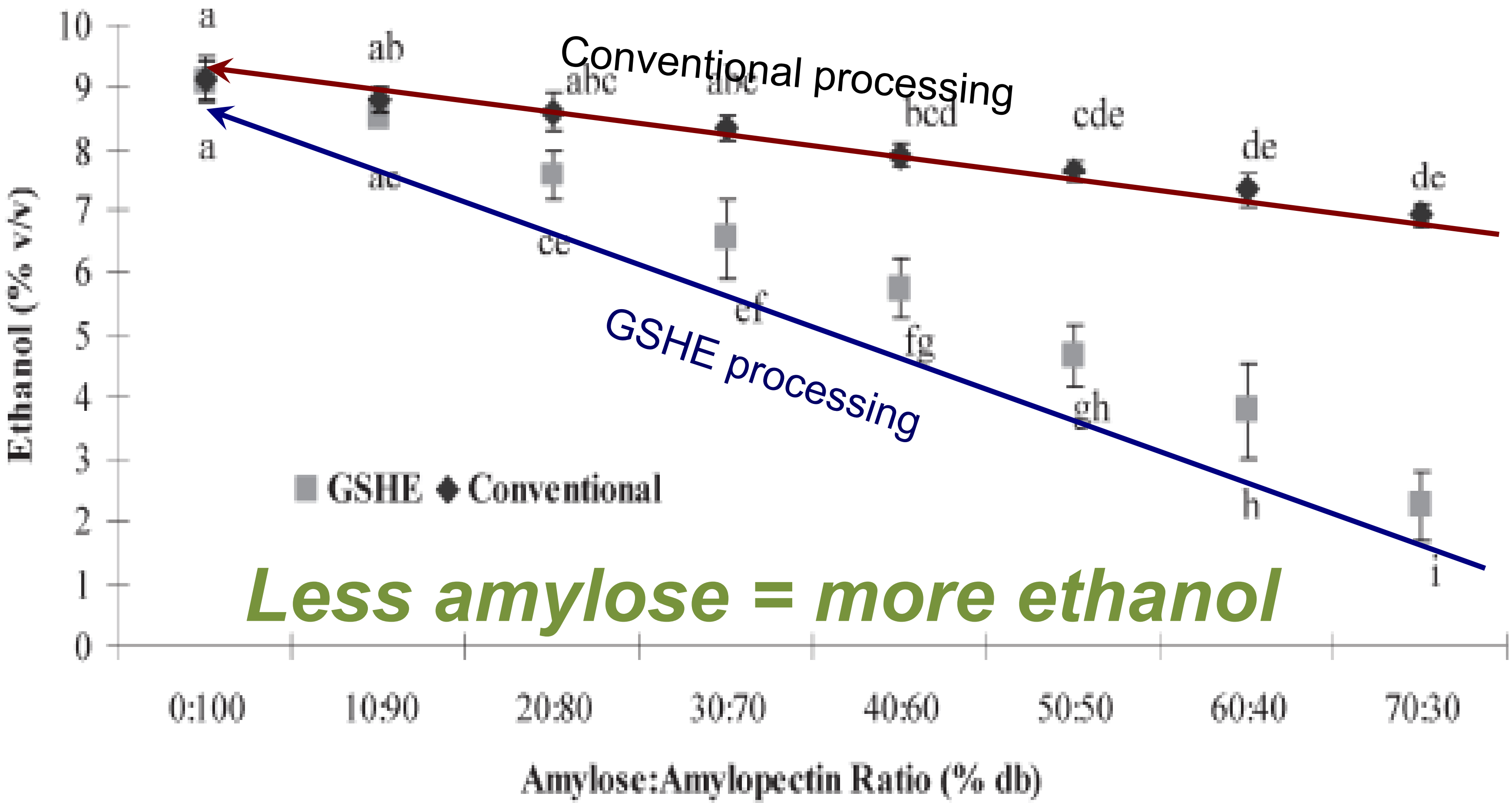

Fig. 1. Ethanol concentration varying with amylose content in starch for GSHE and conventional enzymes processes (data points followed by same letters are not different ($p \leq 0.05$)).

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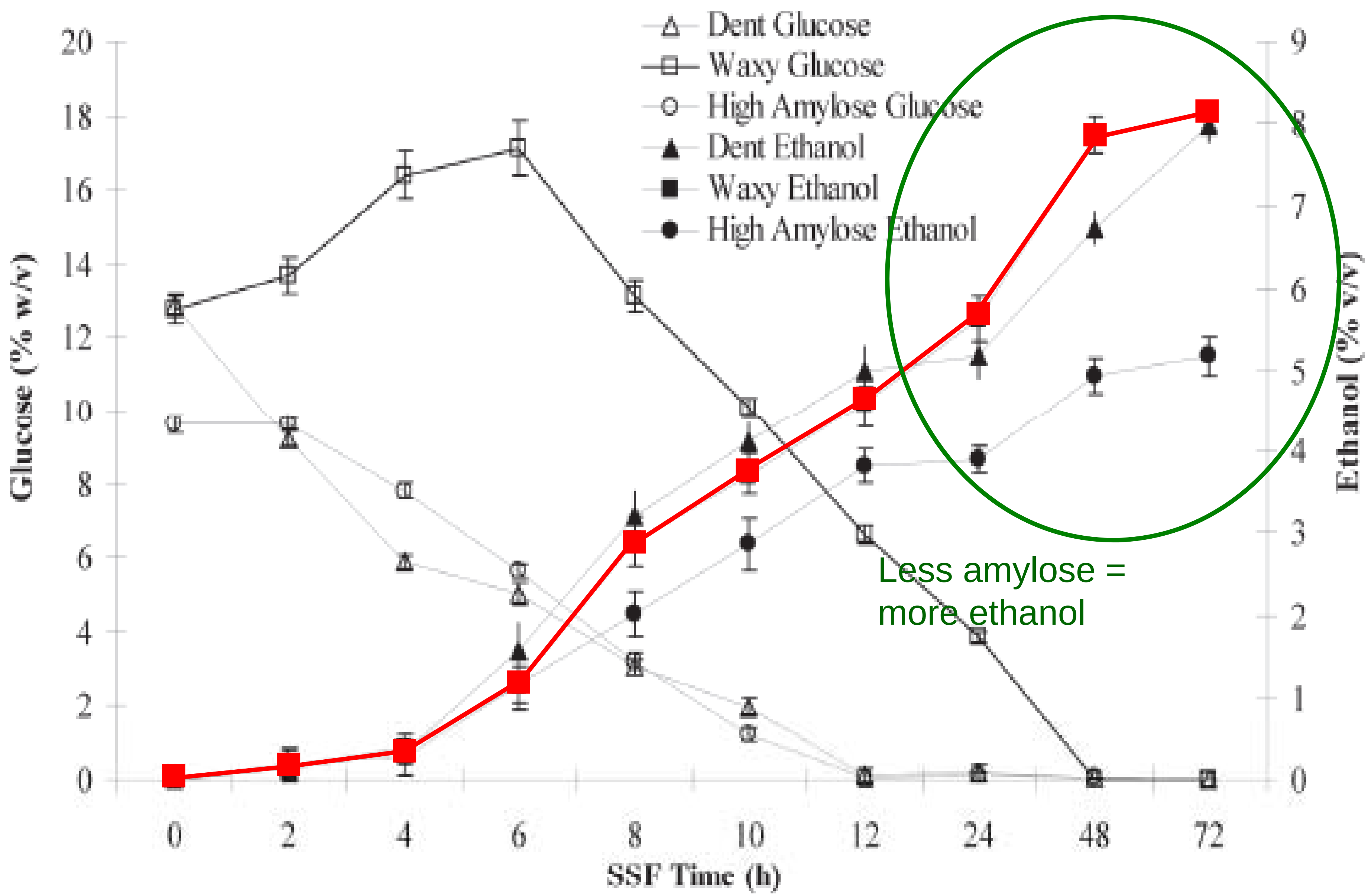

Fig. 7. Ethanol and glucose profiles during simultaneous saccharification and fermentation (SSF) for the conventional enzyme process for dent, waxy and high amylose maize treatments.

Cassava "Novel" traits

Fermentability:

assess their potential in bio-ethanol, bio-plastics, sweeteners

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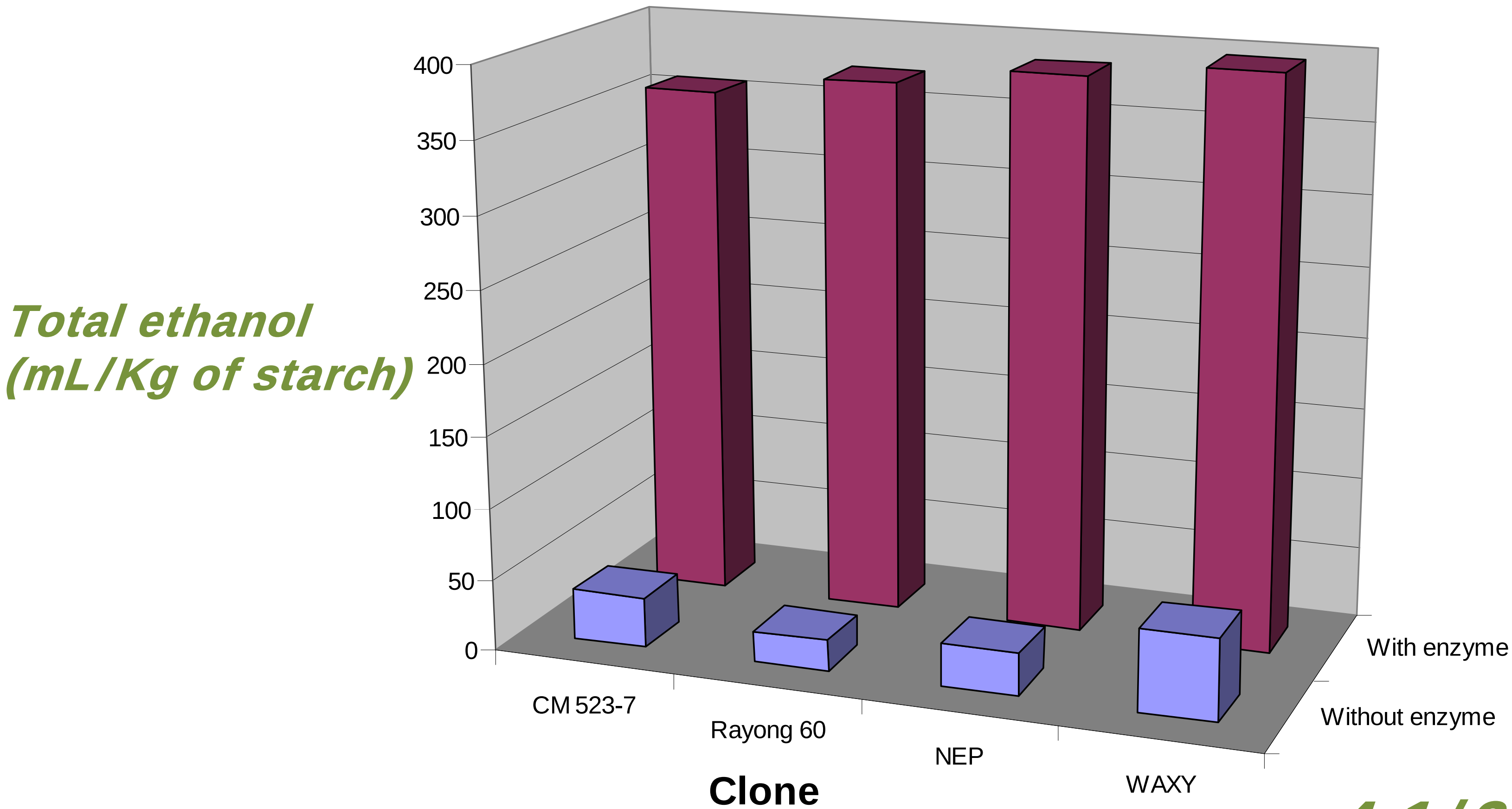
Cassava starch fermentation: with and without starch



4 1/3 days

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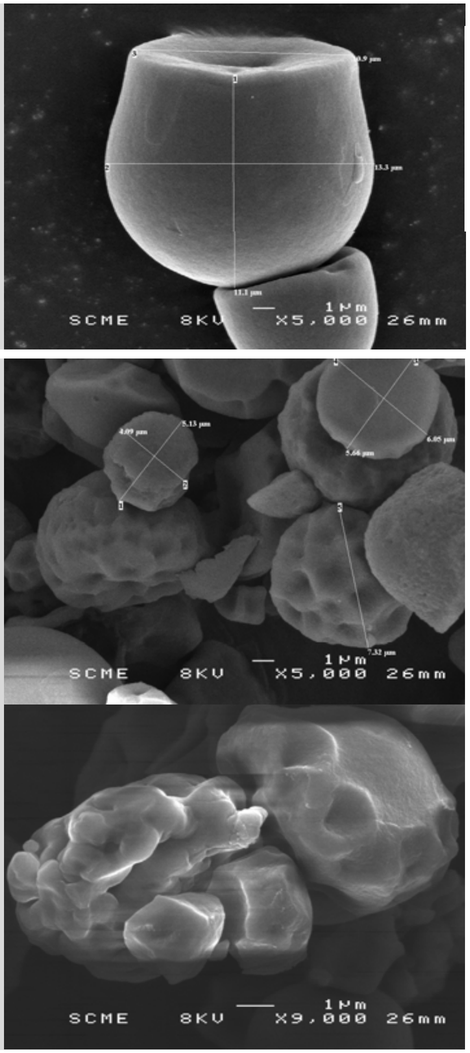
Small granule/high amylose

Normal Starch

Small granule Starch



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2008, 56, 7215–7222 7215

Induction and Identification of a Small-Granule, High-Amylose Mutant in Cassava (*Manihot esculenta* Crantz)

HERNÁN CEBALLOS,*,†,‡ TERESA SÁNCHEZ,† KAY DENYER,|| ADRIANA P. TOFIÑO,‡,§ ELVIA A. ROSERO,‡ DOMINIQUE DUFOUR,†,⊥ ALISON SMITH,|| NELSON MORANTE,† JUAN C. PÉREZ,† AND BRENDAN FAHY||

Centro Internacional de Agricultura Tropical (CIAT), Apdo Aéreo 6713, Cali, Colombia; Universidad Nacional de Colombia-Palmira Campus, Carrera 32 Chapinero, Palmira, Colombia; Corporación Colombiana de Investigación Agropecuaria (CORPOICA-Centro de Investigación Motilonia); John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, U.K.; and Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 73 Rue Jean-François Breton, TA B-95/16, 34398 Montpellier Cedex 5, France

Only two mutations have been described in the literature, so far, regarding starch and root quality traits in cassava. This article reports on an induced mutation in this crop, first identified in 2006. Botanical seed from five different cassava families were irradiated with γ rays. Seed was germinated, transplanted to the field (M_1 plants), and self-pollinated to produce the M_2 generation. Abnormal types regarding starch granule morphology were identified during the single plant evaluation of M_2 genotypes. To confirm these characteristics, selected genotypes were cloned and a second evaluation, based on cloned plants obtained from vegetative multiplication, was completed in September 2007. Two M_2 genotypes presented small starch granules, but only one could be fully characterized, presenting a granule size of 5.80 ± 0.33 μm compared with three commercial clones with granule sizes ranging from 13.97 ± 0.12 to 18.73 ± 0.10 μm and higher-than-normal amylose content (up to 30.1% in cloned plants harvested in 2007, as compared with the typical values for "normal" cassava starch of around 19.8%). The gels produced by the starch of these plants did not show any viscosity when analyzed with the rapid viscoanalyzers (5% suspension), and the gels had low clarity. Low viscosity could be observed at higher concentrations (8 or 10% suspensions). Preliminary results suggest that the mutation may be due to a lesion in a gene encoding one of the isoforms of isoamylase (probably *isa1* or *isa2*).

KEYWORDS: Amylose; amylopectin; granule size; mutation breeding; isoamylase

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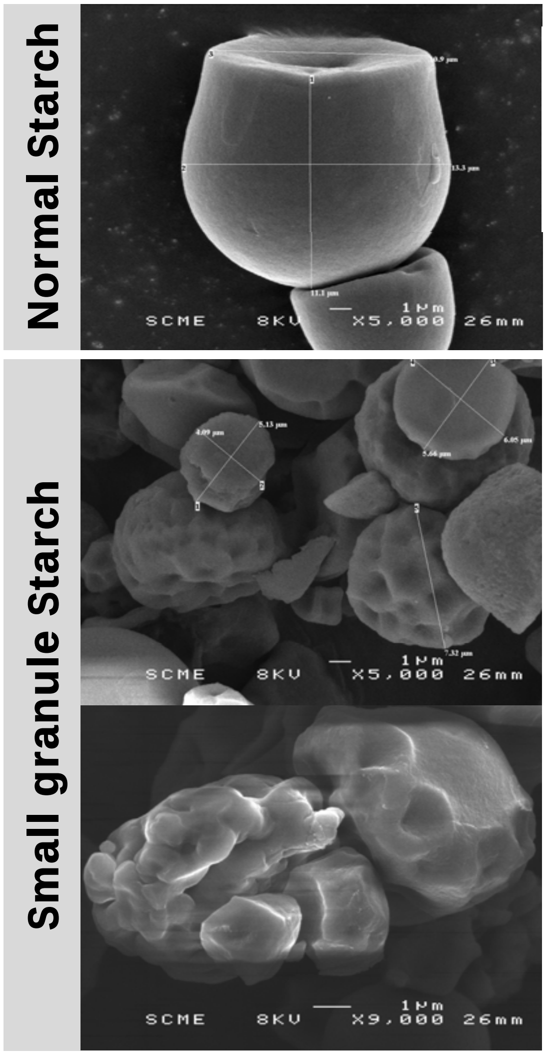
Small granule/high amylose



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2008, 56, 7215–7222 7215

Induction and Identification of a Small-Granule, High-Amylose Mutant in Cassava (*Manihot esculenta* Crantz)

- ***A small granule and a rough surface facilitate the action of enzymes (less consumption of enzymes, lower costs of conversion).***
- ***But higher amylose content would increase costs....***

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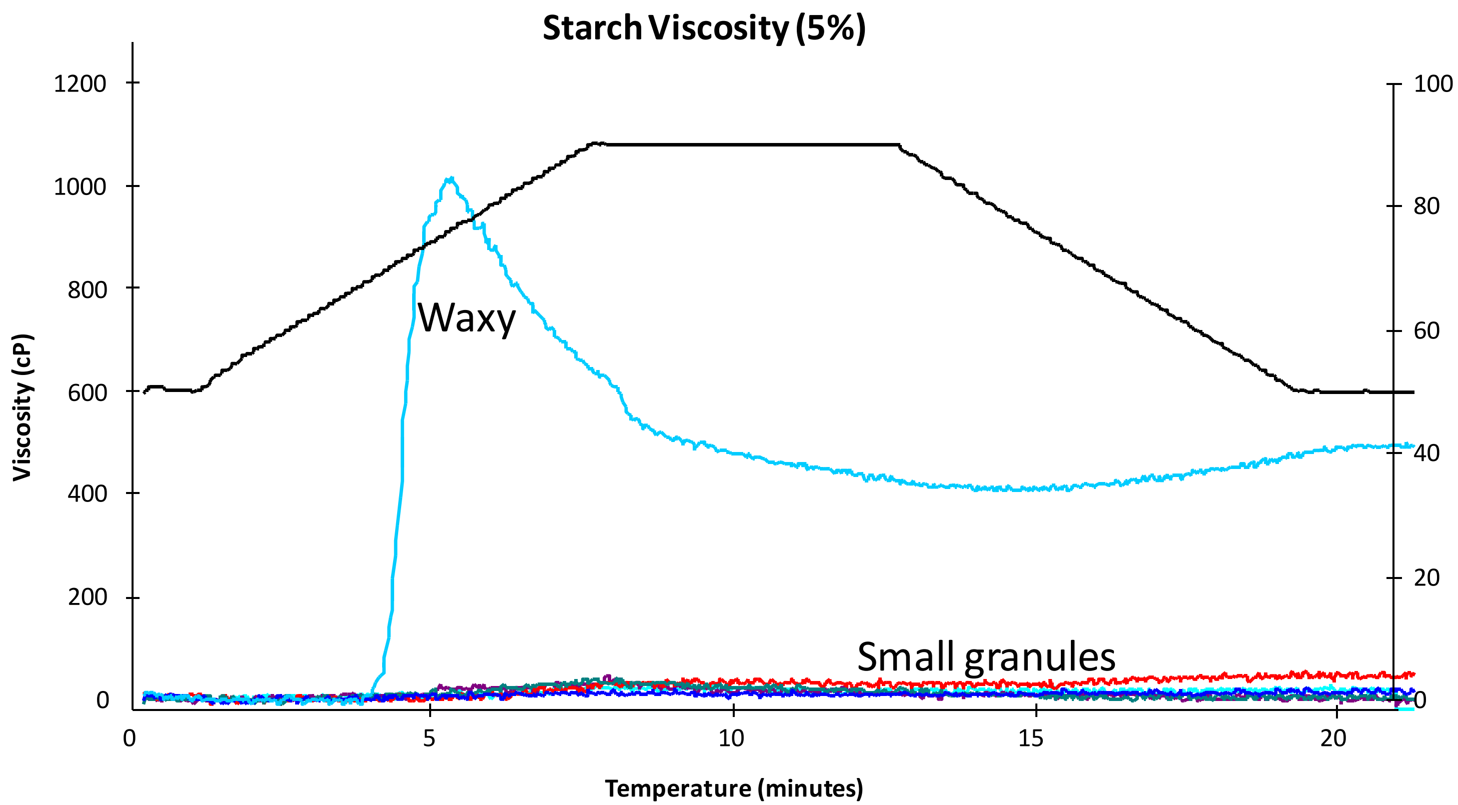
Small granule/high amylose

RVA Amylogram



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Starch-less mutation

Source:
L. Carvalho
EMBRAPA
Brazil

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Bio-ethanol production

Bio-ethanol production

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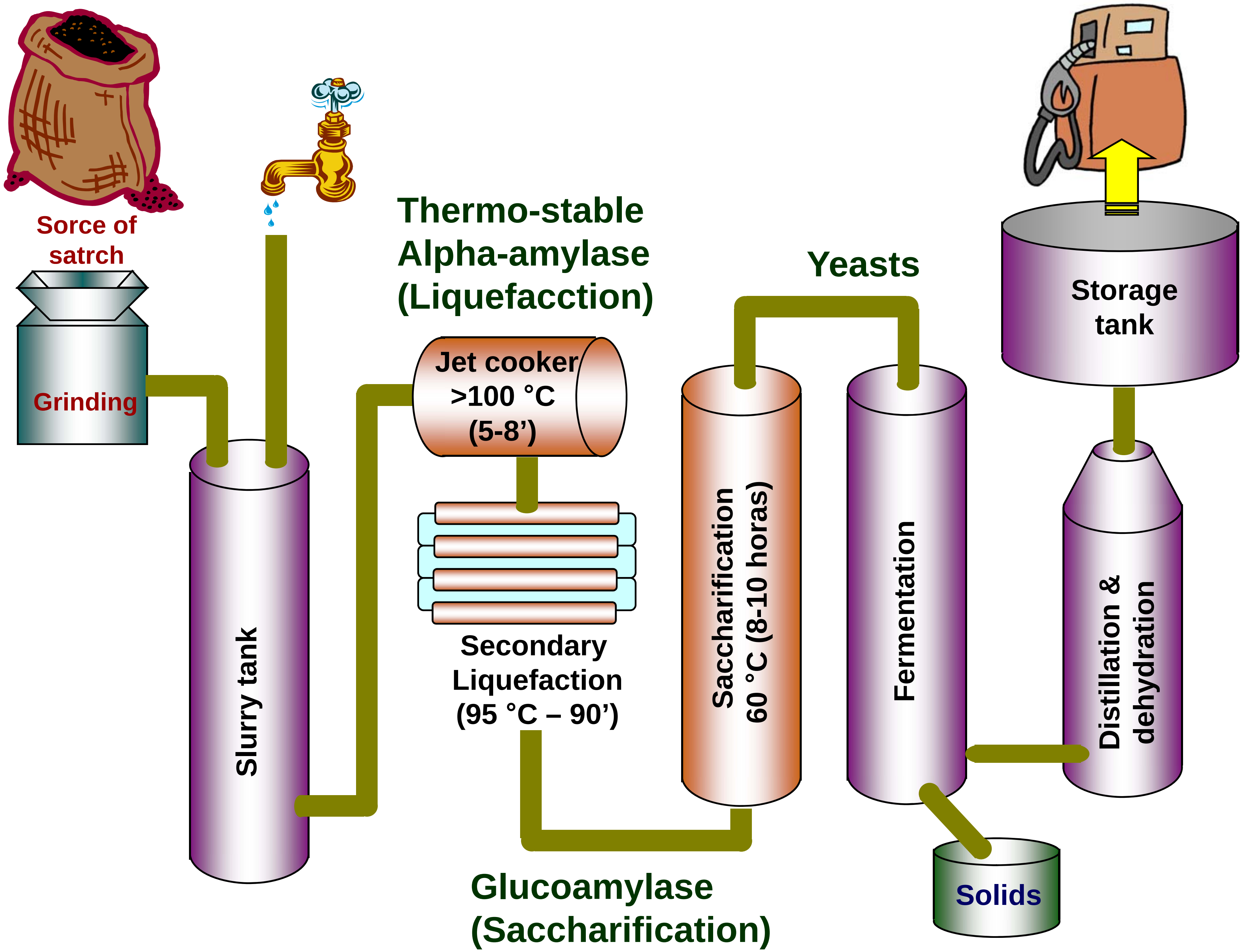
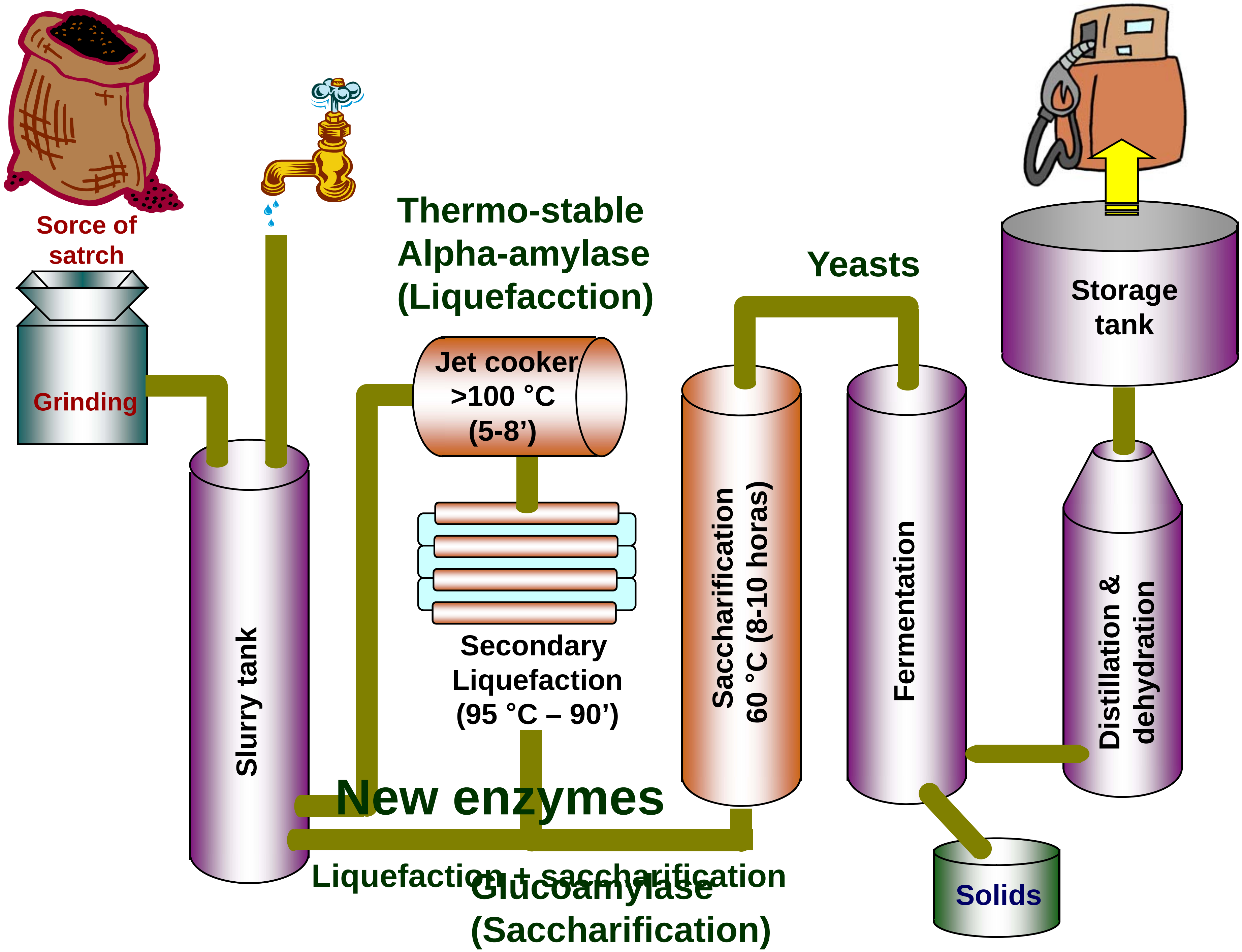
Ethanol from corn or cassava is more expensive because starch need to be degraded to the equivalent of sugar cane juices

Ethanol

Maize or Cassava



Sorce of satrch
Grinding
Slurry tank
Thermo-stable Alpha-amylase (Liquefacction)
Jet cooker >100 °C (5-8')
Secondary Liquefaction (95 °C – 90')
Saccharification 60 °C (8-10 horas)
Yeasts
Fermentation
Storage tank
Distillation & dehydration
Solids
Glucoamylase (Saccharification)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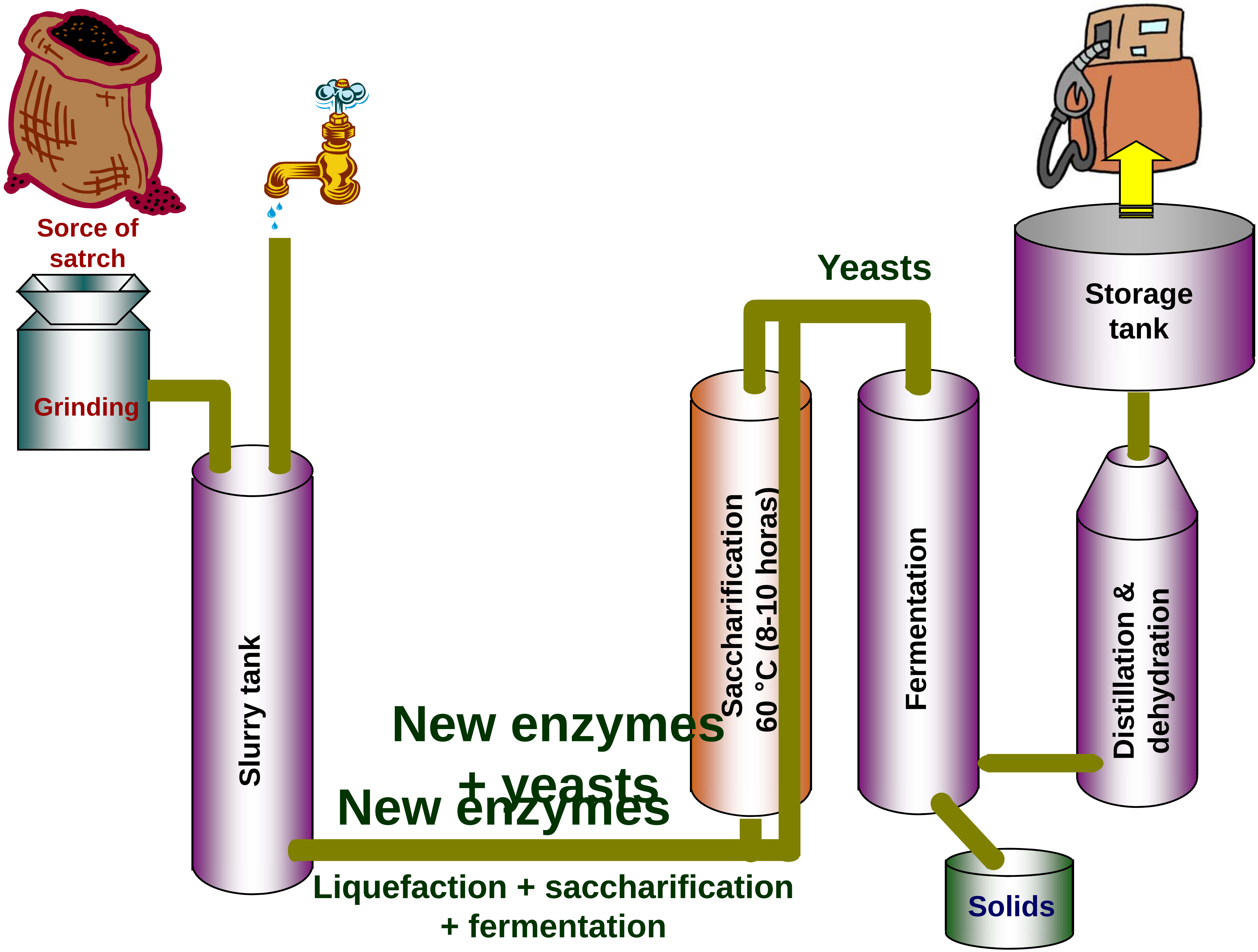
Secondary Liquefaction (95 °C – 90')
Yeasts
Saccharification 60 °C (8-10 horas)
Fermentation
Storage tank
Distillation & dehydration
Solids
New enzymes
Liquefaction + saccharification
Glucoamylase (Saccharification)


Sorce of satrch
Grinding
Slurry tank
New enzymes + yeasts
New enzymes
Saccharification 60 °C (8-10 horas)
Yeasts
Fermentation
Solids
Distillation & dehydration
Storage tank
Liquefaction + saccharification + fermentation


Storage tank
Distillation & dehydration
Solids
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Medium throughput fermenters

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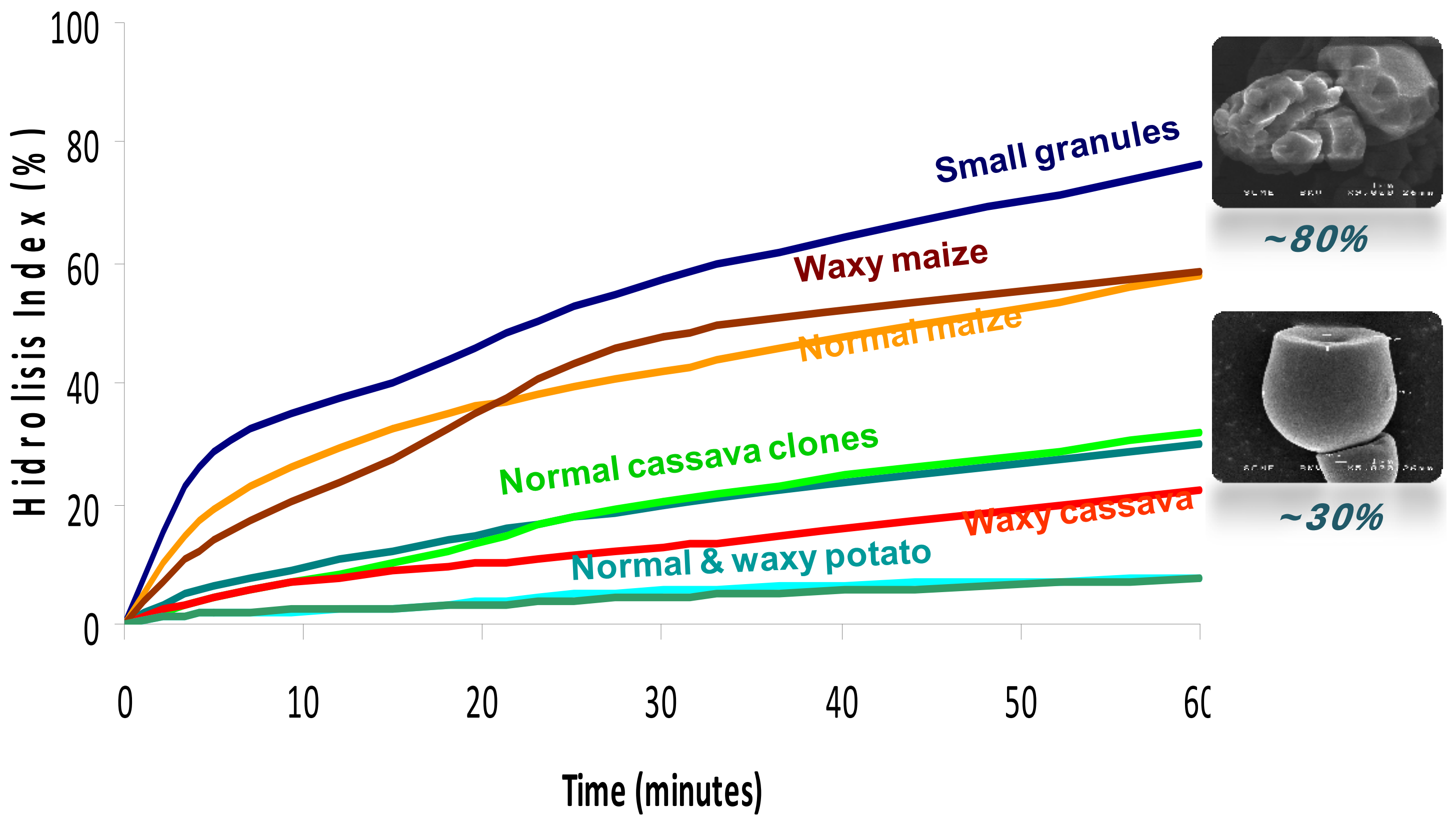
Digestion rate of different cassava starches (1.0 ml of pacreatic α-amilase) pH 6.9 at 37 ℃



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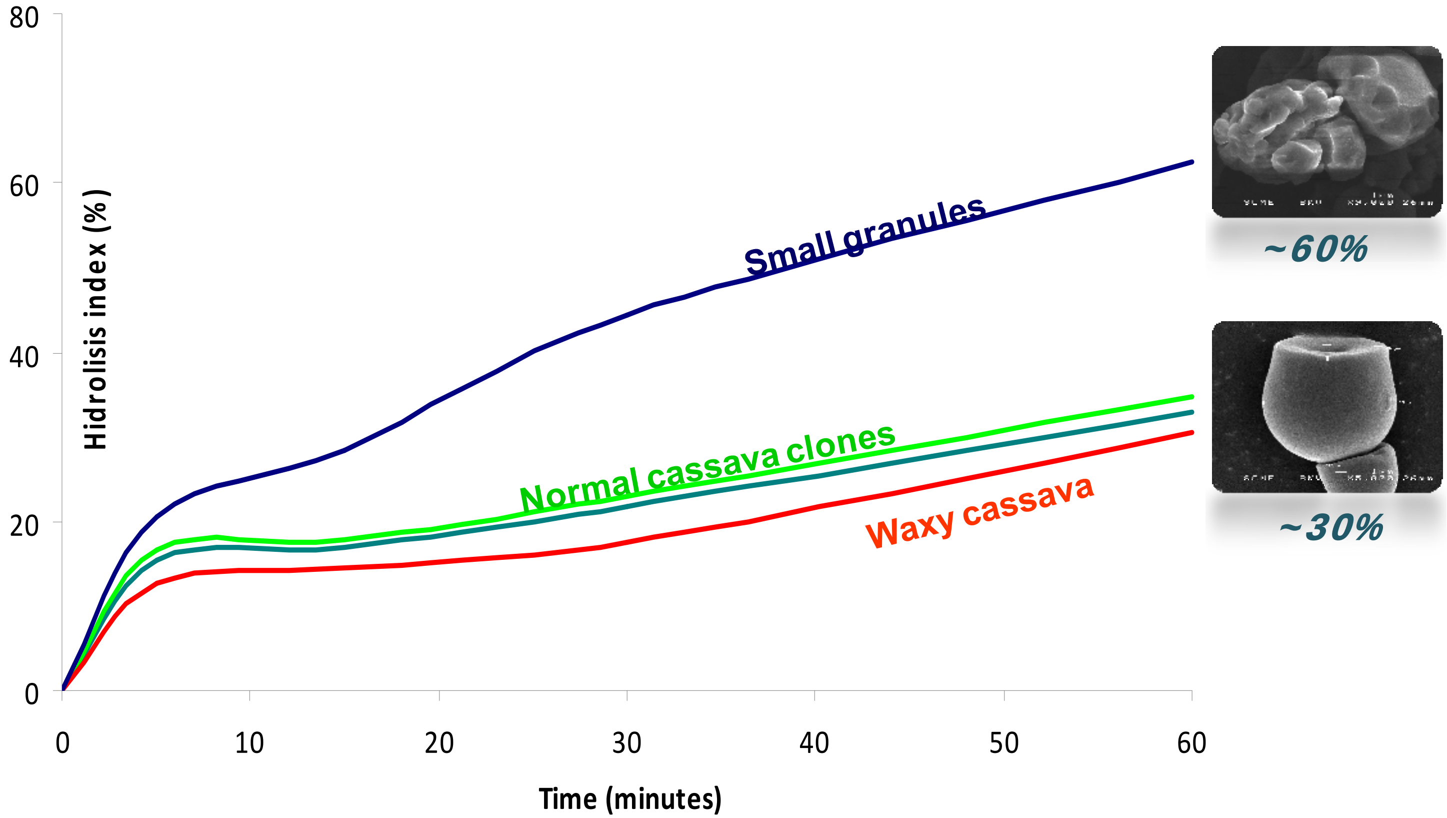
Digestion rate of different cassava starches (0.5 ml of Stargen™ 2) pH 4.0 at 37°C



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Root processing vs. quality

- Starch degrading enzymes and yeast are being improved.
- The process to convert starch into ethanol constantly changes.
- As in maize, there are genetic differences in cassava for ethanol production (small starch granule).
- We are in a unique position to analyze the best germplasm – processing method to maximize economic benefit and reduce negative impact on the environment.
- What is the potential of "sugary" cassava?

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Cassava Bio-ethanol perspective

- Cassava is a competitive raw material for bio-ethanol production in Asia (Thailand, China, Vietnam, Indonesia?, Australia?)
- A large % of the ethanol production cost is constitute by the enzyme and yeast.
- Advances in microbiology and enzymology can significantly reduce ethanol production cost from starches

Cassava Bio-ethanol prespective

- *There are clones with low dry matter content but maximum productivity per hectare that can now be used in ethanol production*

- *Different mutants could reduce costs of conversion from root to ethanol (including "sugary"?)*

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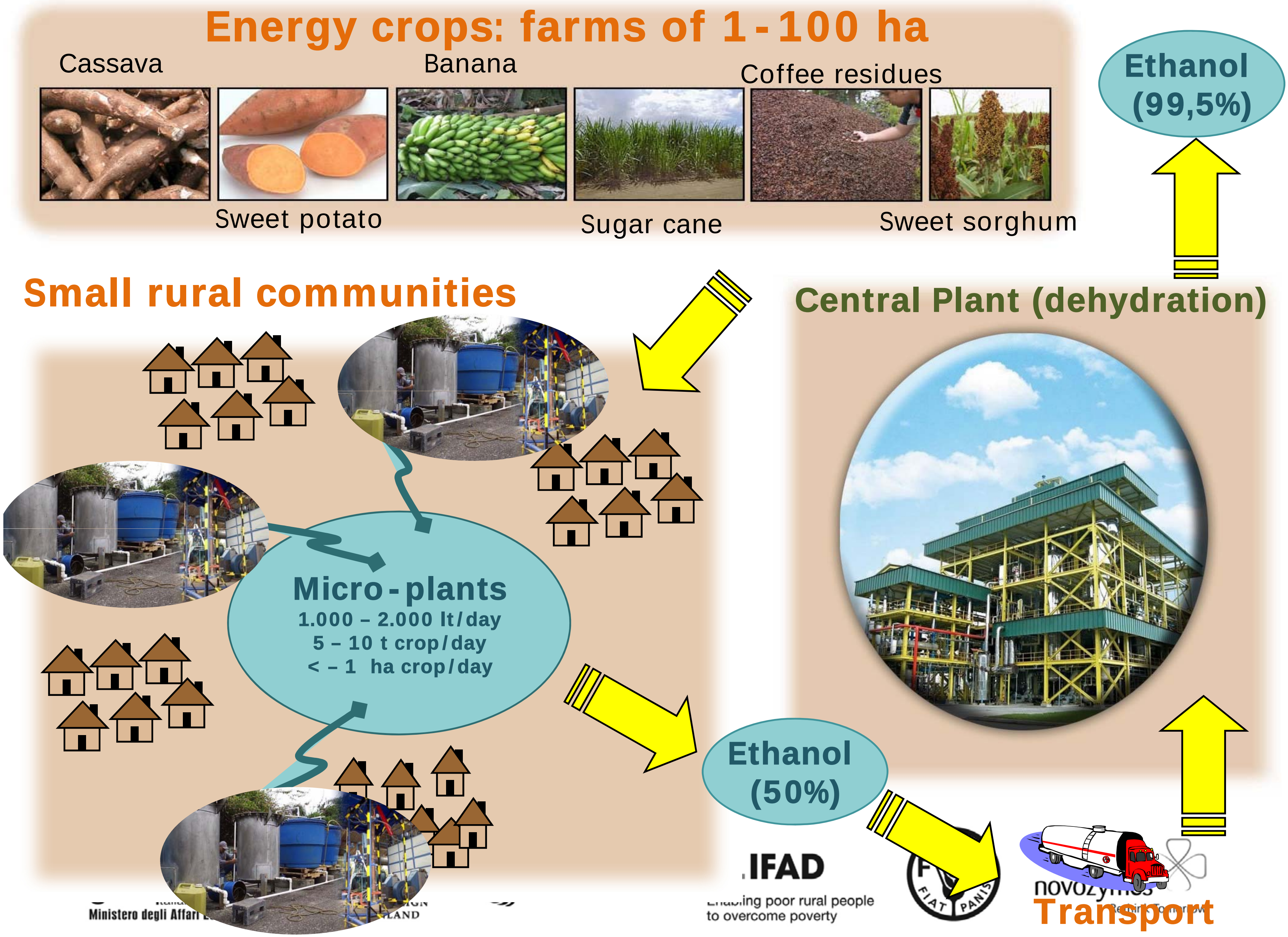

Energy crops: farms of 1-100 ha
Cassava
Banana
Coffee residues
Sweet potato
Sugar cane
Sweet sorghum
Ethanol (99,5%)
Small rural communities
Central Plant (dehydration)
Micro-plants
1.000 – 2.000 lt/day
5 – 10 t crop/day
< – 1 ha crop/day
Ethanol (50%)
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Transport

Cassava Bio-ethanol perspective

- For ethanol production a key issue is the continuous supply of feedstock all year round.
- Processing of fresh roots (low dry matter?) at harvest time and dried chips during off-season is one potential alternative.

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Cassava Bio-ethanol perspective

- Combining feedstock from different crops. For instance, cassava/sweet-sorghum has proved advantageous.
- We need to further analyze the by-products and their potential use for animal feeding.

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Thank you

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